

The unique properties of IgG4 and its roles in health and disease

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Abstract

IgG4 is the least abundant subclass of IgG in human serum and has unique functional features. IgG4 is largely unable to activate antibody-dependent immune effector responses and, furthermore, undergoes Fab (fragment antigen binding)-arm exchange, rendering it bispecific for antigen binding and functionally monovalent. These properties of IgG4 have a blocking effect, either on the immune response or on the target protein of IgG4. In this Review, we discuss the unique structural characteristics of IgG4 and how these contribute to its roles in health and disease. We highlight how, depending on the setting, IgG4 responses can be beneficial (for example, in responses to allergens or parasites) or detrimental (for example, in autoimmune diseases, in antitumour responses and in anti-biologic responses). The development of novel models for studying IgG4 (patho)physiology and understanding how IgG4 responses are regulated could offer insights into novel treatment strategies for these IgG4-associated disease settings.

Sections

Introduction

Development of an IgG4 response

Structure and function of IgG4


Physiological roles of IgG4

IgG4-dependent pathology

IgG4-related diseases

Future directions

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Introduction

Humoral (antibody-mediated) immune responses are important for protection against pathogen invasion but can also cause disease. Antibodies recognize and bind specific structures of pathogens through their Fab (fragment antigen binding) arms. In addition to mediating direct neutralization of the pathogen, this opsonization of pathogen structures can result in the activation of various immune effector pathways through the Fc (fragment crystallizable) region of antibodies. The antibody Fc region interacts with Fc receptors on immune cells such as macrophages, neutrophils and natural killer cells, resulting in antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). The antibody Fc region can also interact with the complement system, resulting in antibody-dependent complement deposition, which further primes pathogens for cellular uptake and destruction. The ability of an antibody to elicit these immune responses depends on the type of Fc tail and modifications thereof (for example, glycosylation)¹. In humans, five classes of antibody are recognized based on their Fc tail: IgM, IgD, IgE, IgA and IgG. IgM antibodies are produced in the early stages of a primary, adaptive immune response. IgM and IgD form the B cell receptors (BCRs) on naive B cells. The primary IgM response can be followed by a second, long-lasting wave of IgG or IgA antibodies, the latter being involved particularly in mucosal immune responses. IgE probably evolved as a defence against parasitic worms and is also involved in various allergic diseases. Such mature antibody responses are produced by antigen-stimulated B cells that have undergone a process known as class-switch recombination, in which a B cell rearranges its DNA to produce another class of antibody with the same specificity. B cells can undergo repeated rounds of class-switch recombination until the DNA has been recombined using the most 3' Fc tail gene segment (Fig. 1).

Four subclasses of IgG exist, numbered according to the order of abundance. IgG1 has the largest relative contribution to total IgG, followed by IgG2 then IgG3 and IgG4. Although IgG4 is the least abundant IgG subclass overall, specific responses can be dominated by IgG4, often associated with chronic or repeated antigen exposure. IgG4 has a unique set of properties compared with the other IgG subclasses that has led to IgG4 being widely regarded as an anti-inflammatory, 'benign' antibody that may have beneficial functions in allergic disease (Table 1). However, evidence is accumulating that IgG4 also has a pathogenic role in a range of diseases. Research in the past decade has shown that IgG4 can have detrimental roles in IgG4 autoimmune diseases (IgG4-AIDs), in tumour immunology and in IgG4-related diseases (IgG4-RDs). The IgG4-AIDs and IgG4-RDs are chronic conditions and for most patients no cure currently exists. Moreover, the increasing use of biological therapies warrants a better understanding of why certain drugs elicit IgG4 anti-drug responses that limit their efficacy.

In this Review, we highlight how the unique structural and functional characteristics of IgG4 contribute to disease onset and progression in these settings. Furthermore, we provide an overview of our current understanding of how IgG4 responses are regulated. By understanding these processes, future therapeutic strategies could be shaped to prevent pathogenic IgG4 responses or induce beneficial IgG4 responses. Although we appreciate that most antibody responses involve a range of different (sub)classes², in this Review we focus on IgG4-associated diseases that have a predominant IgG4 (antigen-specific) response.

Development of an IgG4 response

The production of IgG4 requires that B cells undergo class-switch recombination (Fig. 1). A direct switch from IgM to IgG4 can occur,

but indirect switching through, for example, IgG1 is also – at least theoretically – possible. Although direct evidence is lacking, in BCR repertoire analyses only limited clonal overlap between IgG1 and IgG4 responses has been observed, which suggests that indirect switching to IgG4 via IgG1 is a minor route *in vivo*³. In keeping with this, *in vitro*, naive IgM⁺ B cells readily switch towards IgG4 production⁴, whereas IgG1⁺ memory B cells do not⁵. Interestingly, substantial clonal overlap between IgG4 and IgA2 was observed, which may reflect that common food antigens often induce both IgG4 and IgA2 responses. This could indicate either that there are similar requirements for the development of IgA2 and IgG4 responses or that substantial sequential class-switching from IgG4 to IgA2 occurs. Class-switching from IgE to IgG4 is not possible owing to the order of class-switch elements in the genome (the heavy-chain constant region segments for IgE being downstream (3') of those for IgG4). Therefore, the allergen-specific IgG4 that is induced by specific immunotherapy in patients with IgE-mediated allergic disease must be derived from either precursor B cells capable of switching to both IgE and IgG4 (for example, non-switched or IgG1⁺ memory B cells) or newly recruited (naive) B cells⁶.

Class-switching towards IgG4 is mostly associated with T helper 2 (T_H2) cell responses. The type 2 cytokines IL-4 and/or IL-13 are important for the induction of IgG in general, but class-switching to IgG4 may more strictly depend on these cytokines than does class-switching to IgG1 (ref. 7). IL-10 and regulatory T (T_{reg}) cells may also skew the antibody response towards IgG4 (relative to IgE and, possibly, also IgG1)⁶ (Fig. 1). However, the role of IL-10 is not fully clarified, and studies *in vitro* have yielded conflicting results depending on, amongst other factors, which cell types are present in addition to naive B cells⁴ (Box 1).

IgG4-switched B cells have similar potential for terminal differentiation towards antibody-secreting cells to that of IgG1-switched B cells; hence, limitations in the development of an IgG4 antibody response are not owing to intrinsic limitations of IgG4⁺ B cells⁵. However, IgG4-switched B cells differ phenotypically from IgG1-switched B cells in several aspects^{5,7}. In particular, they have an altered chemokine receptor profile with lower levels of expression of CXCR3, CXCR4, CXCR5, CCR6 and CCR7 – chemokine receptors involved in germinal centre reactions and the generation of long-lived plasma cells. In the circulation, numbers of IgG4⁺ B cells reflect serum IgG4 concentrations⁵, and their levels follow similar patterns throughout life⁸. IgG4⁺ cell numbers in blood are low compared with IgG1⁺ cells and have a relatively low abundance in secondary lymphoid organs⁷. Furthermore, IgG4 production by antibody-secreting cells can be markedly shorter lived than for other IgG subclasses, requiring continuous input from newly differentiating B cells. Indeed, rituximab (anti-CD20) therapy for B cell depletion has been shown to be particularly beneficial in autoimmune diseases characterized by pathogenic IgG4 (auto)antibodies^{9–11}.

During infancy, the proportion of IgG4 in circulation rises slowly with age¹², and IgG4 titres generally continue to increase throughout life until the fifth decade, after which a small gradual decline is observed⁸. Serum levels of IgG4 show great variation in the healthy population, although intra-individual levels are generally stable¹³. One of the hallmarks of most IgG4 responses is that they develop slowly over time for reasons that are not well understood⁶. Prolonged or repeated exposure to antigen seems to be a necessary – but not sufficient – factor for the development of an IgG4-dominated response. For example, individuals hyperimmunized with tetanus toxoid have an IgG1-dominated response with little IgG4 despite repeated antigen exposure¹⁴, whereas individuals repeatedly vaccinated with SARS-CoV-2 mRNA were shown, in some cases, to have increased proportions of IgG4 after a third

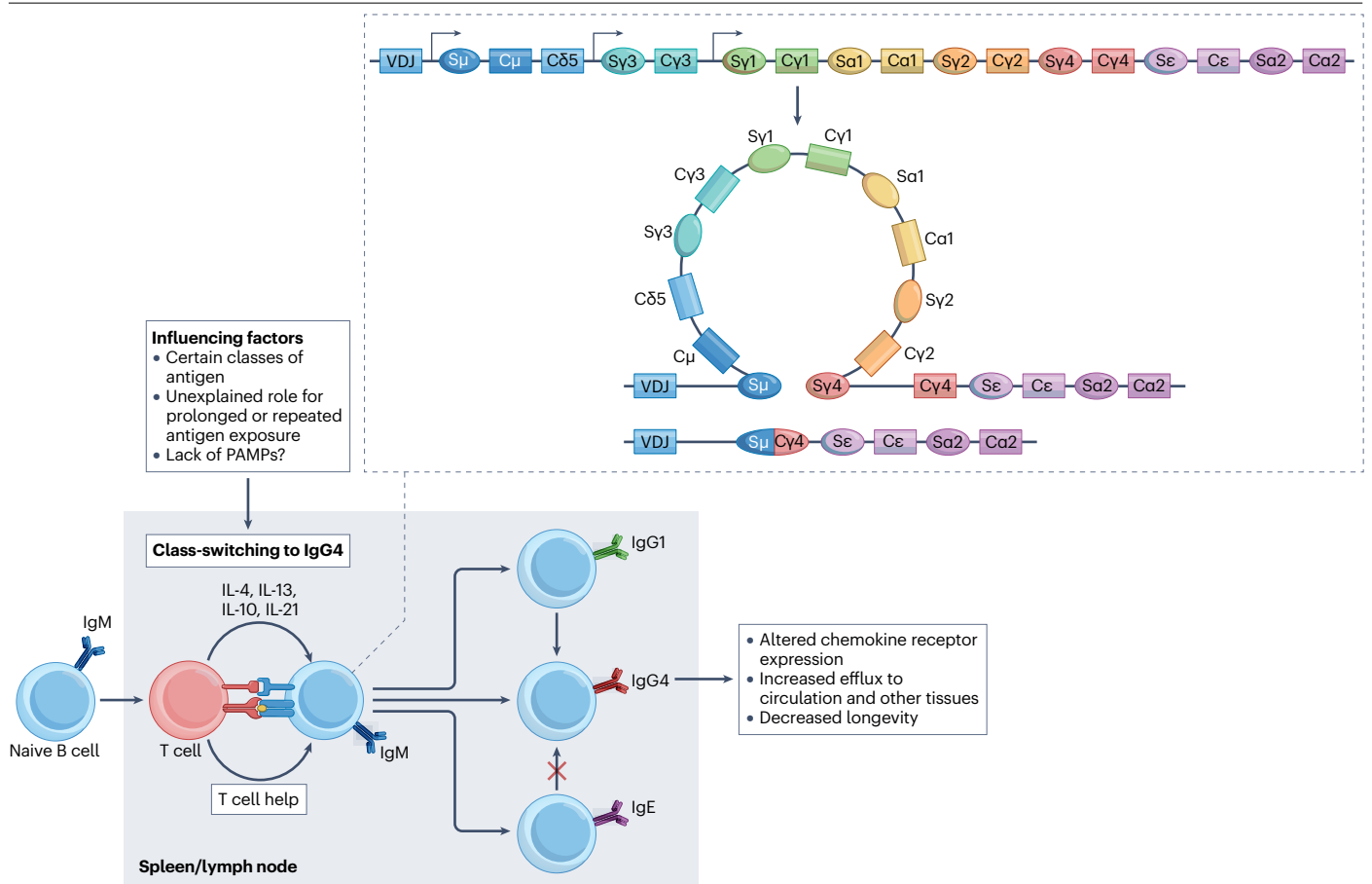


Fig. 1 | The onset and evolution of IgG4 responses. IgG4 responses are most often observed upon repeated or prolonged exposure to certain classes of antigen, including food antigens, parasites, therapeutic proteins and certain autoantigens. IgG4 responses are T cell dependent, with a role for IL-4 and/or IL-13, IL-10, IL-21 and regulatory T (T_{reg}) cells in their induction. The absence of certain pathogen-associated molecular patterns (PAMPs) associated with viral or bacterial infection may favour IgG4 development for unknown reasons. Class-switching to IgG4 may occur directly from IgM⁺ B cells, or indirectly via IgG1⁺ intermediates, but the relative contribution of these routes is not clear. Although IgG4 can be induced by specific immunotherapy to alleviate IgE-mediated allergic symptoms, the shift towards an IgG4-dominated antibody response is

not the result of IgE⁺ B cells class-switching towards IgG4, as this is not possible in light of the class-switch order of immunoglobulin heavy-chain constant region segments in the genome (see inset; once a segment has been removed by prior class-switching, a B cell cannot express it anymore). IgG4⁺ B cells have phenotypic traits that are distinct from those of IgG1⁺ B cells, including an altered chemokine receptor profile, with lower levels of expression of CXCR3, CXCR4, CXCR5, CCR6 and CCR7 – chemokine receptors involved in germinal centre reactions and the generation of long-lived plasma cells – which therefore likely results in the increased efflux of IgG4⁺ B cells from germinal centres and influx into other tissues, and might also have a role in the overall reduced longevity of IgG4-switched B cells. Inset box adapted from Fig. 1 of ref. 157, Springer Nature Limited.

vaccination, requiring at least 6 months to develop¹⁵. IgG4 is not commonly part of the antibody response to bacterial or viral infection. The range of situations in which specific IgG4 is or can be a dominant factor is wide and includes responses to allergens, therapeutically administered proteins, autoantigens and helminth infections. With the exception of helminths, the absence of an infectious agent seems to be a common feature of IgG4 responses and it is tempting to speculate that the absence of certain danger signals such as pathogen-associated molecular patterns (PAMPs) is a prerequisite for B cells to differentiate towards IgG4-secreting cells in vivo. Indeed, the proportion of IgG4 antibodies was smaller in individuals receiving whole-cell pertussis vaccine than in individuals receiving acellular pertussis vaccine (although IgG4 was only a small fraction of the total IgG response even in the latter)¹⁶.

Structure and function of IgG4

Despite the high levels of homology between human IgG subclasses, each subclass has a specific set of functional characteristics owing to particular structural features¹ (Table 1). IgG4 is unique in that it has a lesser affinity than other IgG subclasses for many effector molecules, such as Fc receptors and complement, and also because of structural features that affect interactions through the Fab region, such as Fab-arm exchange and a greater propensity for acquiring glycosylation in the variable domains^{4,17} (Fig. 2).

Fc-dependent effector functions

IgG4 differs in several key amino acid positions from the other IgG subclasses, resulting in a modified binding pattern to Fcγ receptors (Table 1 and Fig. 2). In particular, relative to IgG1, the amino acid changes

Table 1 | IgG subclasses and their functional characteristics

Feature	IgG1	IgG2	IgG3	IgG4
Relative abundance (% of total IgG in serum)	60	32	4	4
Fcγ receptor binding	+++	+/-	+++	+/- ^a
C1q binding	++	+	+++	+/-
Hinge length (number of amino acids)	15	12	Up to 62	12
Inter-heavy-chain disulfide bonds	2	4	11 (depending on allotype)	2
Fab-arm exchange	No	No	No	Yes
Fc glycosylation	Yes	Yes	Yes	Yes
Fab glycosylation	Yes	Yes	Yes	Greater than other subclasses

Fab, fragment antigen binding; Fc, fragment crystallizable. ^aIgG4, similarly to IgG1, can interact with the inhibitory receptor FcγRIIB, which, owing to the limited interaction of IgG4 with activating Fcγ receptors such as FcγRIII (the most highly expressed FcγR in humans), steers overall cellular responses towards inhibition.

at L234F, A327G and P331S in IgG4 are implicated in effects on Fcγ receptor binding^{18–20}. Binding to most Fcγ receptors is reduced for IgG4 (although not completely abrogated), resulting in IgG4 having poor ADCC activity^{21,22} and, probably, also ADCP activity (although this has not been studied in detail). Interestingly, binding of IgG4 to the inhibitory receptor FcγRIIB is not affected, which skews Fcγ receptor signalling induced by IgG4 away from cellular activation and towards inhibition. In the case of IgG1, interaction with the activating receptor FcγRIIIa is markedly increased if the conserved Fc glycan does not contain the core fucose moiety. This is also true for IgG4, and an afucosylated variant of IgG4 was found to induce ADCC, albeit still less efficiently than did IgG1 (ref. 23). However, naturally occurring afucosylated antibody responses seem to be restricted to antiviral responses or alloimmunity to blood cells and platelets, which normally do not have high levels of IgG4 (refs. 1,24). The limited signalling of IgG4 through activating Fc receptors might attenuate the impact of certain antibody responses; for example, it has recently been shown in a mouse model of thrombotic thrombocytopenic purpura that recombinant IgG1 antibodies to ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13) are more pathogenic than their IgG4 counterparts in an Fcγ-dependent manner²⁵.

In addition to the ‘classical’ Fcγ receptors (FcγRI–FcγRIII), two ‘non-classical’ Fcγ receptors – Fc receptor-like protein 4 (FCRL4) and FCRL5 – have been reported to bind IgG4, albeit weakly^{26,27}. These receptors are mostly expressed on B cells and have been described to either inhibit or enhance BCR signalling, the latter only if CD21 is simultaneously engaged^{21,28}, thereby augmenting or counteracting the role of FcγRIIB. A specific role of IgG4 in this signalling route is as yet unknown.

Furthermore, IgG4 is a poor activator of complement, resulting in a poor capacity for inducing antibody-dependent complement deposition and ADCP. Complement has an important role in clearing pathogens and promoting inflammation, which consequently is limited when IgG4 dominates the antibody response²⁹. This results mainly from reduced binding to C1q, caused mainly by residue S331 of IgG4 (refs. 30,31), which is the counterpart of P331 in IgG1 (the homologous P436S mutation in IgM also markedly affects C1q binding)³².

Nevertheless, some studies suggest that IgG4 can activate complement in specific contexts. For example, artificially enforcing the hexamerization of IgG4 – a process that normally would take place ‘spontaneously’ as part of complex formation of antibody with C1 – results in complement activation by the classical route^{33,34}. This shows that IgG4 has a reduced ability to activate complement but is not completely ‘silent’ in this respect. In patients with membranous nephropathy, all of whom have complement deposition in the kidneys³⁵, IgG4 autoantibodies to phospholipase A2 receptor 1 (PLA2R1) are associated with disease. Recent work indicates a possible role for these IgG4 autoantibodies in complement activation via the lectin pathway, whereby decreased galactosylation levels on the autoantibodies allow for mannose-binding lectin (MBL) binding and complement deposition³⁶. This is in contrast to increased levels of galactosylation promoting IgG1 hexamerization and complement activation by the classical route³⁷, and other studies suggest a pathogenic role of IgG4 autoantibodies to PLA2R1 independent of complement³⁸. Complement activation could be shown in vitro for glyco-engineered recombinant IgG4 antibodies, but only at high antigen density and high antibody concentration, and no contribution of the lectin pathway was observed³⁹.

Fab-arm exchange

Uniquely, serum IgG4 typically does not cross-link antigen⁴⁰. In fact, IgG4 often behaves effectively as a monovalent antibody in the circulation. IgG4 molecules are produced as bivalent, monospecific antibodies but can, subsequently, engage in a process in which half-molecules of IgG4 (heavy chain and light chain) are randomly exchanged with other IgG4 half-molecules, through a process known as Fab-arm exchange¹⁷ (Box 2). This makes most IgG4 molecules in the blood bispesific. Early evidence of this process included the ability of serum IgG4 to cross-link two different allergens⁴¹. However, in many cases, the second antigen specificity of an IgG4 molecule will be irrelevant because the exchange is random, and the resulting antibody will behave as if monovalent. Thus, effective binding and downstream signalling of functionally monovalent IgG4 will require high affinity for antigen, as IgG4 cannot benefit from the accumulated binding strength (avidity) of multiple Fabs with the same specificity⁴². Interestingly, in patients with eosinophilic oesophagitis, very high titres of specific IgG4 to cow milk protein have been observed (together with deposits of IgG4 in the oesophageal wall)⁴³, to such a degree that a substantial portion of IgG4 may still be bivalent in this context^{44,45}; this suggests that the effective monovalency of IgG4 is not absolute but depends on the relative levels of specific IgG4 and total IgG4.

The process of Fab-arm exchange is controlled by redox conditions and can be promoted in vitro by choosing an appropriate redox buffer^{46,47}. Comparing IgG4 with IgG1, two mutations in the latter are required to enable Fab-arm exchange: a P228S mutation in the hinge region allowing for the disulfide bonds that normally connect the heavy chains to be easily broken, and a K409R mutation in the carboxy-terminal domains that results in weaker non-covalent interactions between the heavy chains⁴⁸. Conversely, therapeutic IgG4 monoclonal antibodies often contain a S228P mutation to prevent Fab-arm exchange in vivo⁴⁹.

A major functional consequence of the effective monovalency of the majority of IgG4 in vivo is that it further reduces the ability for signalling, antigen cross-linking and immune activation (Fig. 2). Furthermore, Fab-arm exchange seems to further decrease the limited potential of IgG4 for complement activation³⁹. Therefore, Fab-arm exchange together with the overall reduced ability of IgG4 to activate

Fcγ receptors and complement means that IgG4 is often regarded as a natural type of ‘blocking’ antibody – a high-affinity monovalent binder with limited potential to induce inflammatory responses⁴. Because of these weak effector functions and blocking ability, IgG4 is the second most widely used antibody format for therapeutic monoclonal antibodies, with examples including natalizumab, nivolumab and reslizumab.

Glycosylation

The glycan structure on IgG molecules can vary. Specific glycan profiles have been associated with (patho)physiological conditions, and the exact glycan structure can affect antibody functions such as Fcγ receptor activation and complement activation. This has been investigated in most detail for the conserved N-linked glycans in the Fc region. In general, Fc galactosylation of IgG4 seems to be decreased in pathological conditions^{36,50,51}. Furthermore, N-linked glycans are also present in the variable regions of immunoglobulins to different degrees⁵². Variable domain glycosylation is largely dependent on acquiring mutations that introduce glycosylation motifs during somatic hypermutation, such that specific antibody responses may be highly enriched or depleted for variable domain glycans⁵³. In particular, certain autoantibodies, including those against muscle-specific tyrosine kinase, desmoglein 3 (DSG3) and proteinase 3, are found to be highly glycosylated in the variable domain^{54–56}. Interestingly, IgG4 antibodies in general have increased levels of Fab glycans compared with other IgG subclasses^{51,57}. This feature seems to be associated with the type 2 response-like characteristics of the IgG4 response, as BCR repertoire analysis of both IgG4 and IgE responses showed increased levels of N-glycosylation motifs⁵⁷. The functional consequences of these glycans are not well understood but

may include attenuation of antibody-mediated signalling by engaging lectins such as CD22 (ref. 52), elimination of autoreactivity⁵⁸ or enhancement of BCR signalling⁵⁹. The link between antibody glycosylation and pathogenicity of IgG4 autoantibodies warrants further investigation.

Physiological roles of IgG4

In general, the ‘blocking’ nature of an IgG4 response may be beneficial when it prevents excessive immune activation. In particular, in both allergic responses and parasitic infections, IgG4 responses are beneficial for the host by inducing tolerance and limiting inflammation (Fig. 3).

Allergic responses

Allergy is characterized by hypersensitivity reactions that lead to symptoms including rash, swelling, itching, upper respiratory tract sensitivity and, in severe cases, shock. These reactions result from allergens that trigger IL-13 and IL-4 release by T_H2 cells and subsequent class-switching of B cells to IgE production. In response to FcεRI stimulation by IgE bound to allergen, mast cells and basophils release histamine, cytokines and chemokines, which have effects on the vasculature and tissues to cause the hypersensitivity symptoms. Peripheral tolerance to allergens can be achieved by specific immunotherapy or by regular exposure to them, which may induce allergen-specific IgG4 that can contribute to reducing hypersensitivity reactions by competing with IgE for binding to allergen and by other mechanisms^{60–65} (Fig. 3a). Allergen-specific IgG4 responses have been described for a range of allergies, including to grass and birch pollen, cats, bee venom, peanuts and milk⁴.

In individuals who are allergic, IgG4 can constitute more than 75% of allergen-specific IgG after continuous exposure to antigen⁶⁶. After specific immunotherapy, IgG1 and, in particular, IgG4 allergen-specific

Box 1

In vitro and in vivo models to study IgG4 responses

In vitro models

Model systems to study IgG4 in vitro generally involve either cell cultures or, more recently, organoids. IgG4⁺ memory B cells and IgG4-producing plasma cells can survive for several days to weeks in vitro when supplied with sufficient trophic factors and cytokines including IL-21 and in the presence of other cell types such as stimulated T cells or CD40L-expressing cell lines. These models allow for, for example, isolation of antibody sequences, manipulation of antibody glycosylation or study of B cell receptor (BCR) signalling and recall responses. IgG4⁺ B cells readily differentiate into antibody-secreting cells in vitro, similarly to IgG1⁺ B cells⁵. Furthermore, in vitro class-switching of naive human B cells towards IgG4⁺ B cells has been shown by many groups to proceed readily, in an IL-4-dependent manner (reviewed in ref. 4). Human tonsil organoids are another model system in which to study (de novo) IgG(4) responses¹⁵⁸. These organoids can live for several weeks and produce varying amounts of antibodies, including IgG4.

In vivo models

Studies of IgG4 in commonly used experimental animal models are challenging owing to the lack of a proper homologue of human

IgG4. Mice and rats each have an IgG subclass that, similar to human IgG4, has limited ability to activate complement, but the properties of these antibodies differ significantly from the anti-inflammatory properties of human IgG4 (ref. 4). In addition, the IgG4-equivalent IgG subclasses do not undergo Fab-arm exchange under physiological conditions⁴⁸. Non-human primates (such as cynomolgus monkeys and rhesus macaques) have an IgG4 subclass that has many similarities to human IgG4, and these primates are therefore the favoured model for IgG4-related research¹⁷. Humanized animal models provide another method for studying IgG4 responses. Recently, human IgG4 responses were successfully induced upon IL-10 exposure of immunodeficient NOD-scid IL2r^{null} mice engrafted with human lymphocytes¹⁵⁹. A human IgG4 knock-in mouse model has been developed, but awaits formal peer-reviewed publication¹⁶⁰. Introducing human IgG4 into animal models is an interesting strategy but the extrapolation of results may be limited as Fcγ receptors have not been adjusted in these models, and the responses may therefore differ from the human situation. Although IgG(4) anti-drug responses are highly unwanted in patients, they form an important model to study de novo IgG4 responses in a human context.

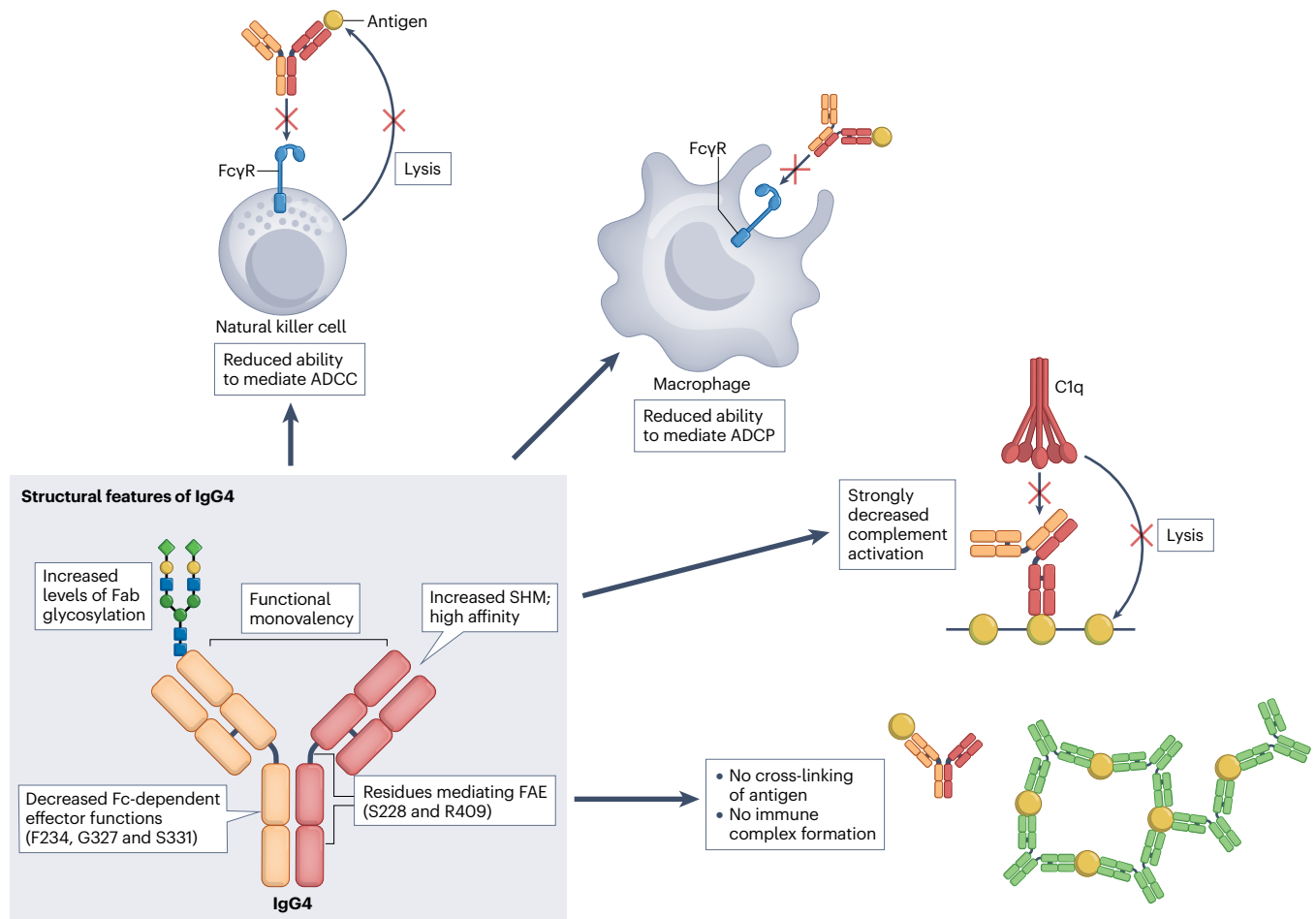


Fig. 2 | Structural and functional characteristics of IgG4. IgG4 has several unique structural features compared with other IgG subclasses, including specific biases in the IgG4 response repertoire (high affinity and increased levels of Fab (fragment antigen binding) glycosylation), functional monovalency (owing to Fab-arm exchange (FAE)) and a reduced ability to induce effector functions mediated by interactions in the Fc (fragment crystallizable) region. Important residues mediating Fab-arm exchange of IgG4 are serine at position 228 (S228) and arginine at position 409 (R409); C1q and Fc receptor binding are particularly reduced by phenylalanine at position 234 (F234), glycine at

position 327 (G327) and serine at position 331 (S331) of IgG4, although residues at other positions may also contribute to the altered binding patterns of IgG4. The functional consequences of these structural features include reduced ability to mediate the Fc-dependent effector functions of antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), a poor ability to activate complement through the Fc domain and interference with immune complex formation through the inability to cross-link antigen. SHM, somatic hypermutation.

responses can increase in the range 10- to 100-fold, with levels starting to increase after 1 month of therapy^{66,67}. An increase in the titre of allergen-specific IgG4 generally correlates with increased tolerance and reduced hypersensitivity symptoms. The protection against symptoms of allergy mediated by IgG4 is thought to be the result of at least three modes of action: blocking the activity of IgE by competing for allergen binding and preventing mast cell and basophil degranulation⁶⁸⁻⁷¹; inhibiting antigen presentation to T cells by IgE on B cells and dendritic cells⁶⁶; and preventing immune complex formation through the functional monovalency of IgG4. The induction of allergen-specific IgG4 responses is thought to result from prolonged exposure to the allergen and increased production of IL-10 (refs. 62,68). IL-10 not only induces T cell tolerance but also regulates antibody production, resulting in increased IgG production relative to IgE production^{72,73}. IgE-induced CD4⁺ T cell activation is a very potent route to maintain

chronic inflammation. Antigen presentation to T cells via B cells and dendritic cells may be facilitated by IgE. By blocking these effects, IgG4 halts this positive-feedback loop, which limits IgE production and puts a brake on the inflammatory response.

Parasitic infections

IgG4 responses can also occur during parasitic infection (Fig. 3b). The host usually develops a broad B cell-mediated and T cell-mediated immune response against the parasite. In an attempt to evade the host immune response, the parasite stimulates production of cytokines such as IL-10 and induction of T_{reg} cells. As a consequence, in a subset of patients, the anti-parasite B cell response may undergo class-switching towards IgG4. IgG responses can consist of up to 90% IgG4 in these asymptomatic patients⁷⁴. A dual role for IgG4 in these infections can be envisaged. On the one hand, the ratio of IgG4 to IgE

may positively correlate with asymptomatic infection, potentially by preventing ongoing inflammation and damage to host tissues, for example in the case of *Brugia malayi* infection⁷⁴. On the other hand, the ratio of specific IgG4 to IgE may correlate with the intensity

of infection⁷⁵ and might represent escape of the parasite from host immunity. Indeed, histamine release by IgE-opsonized basophils from patients with filariasis challenged with filarial antigen was blocked by patient-derived IgG4, and histamine release inversely correlated with

Box 2

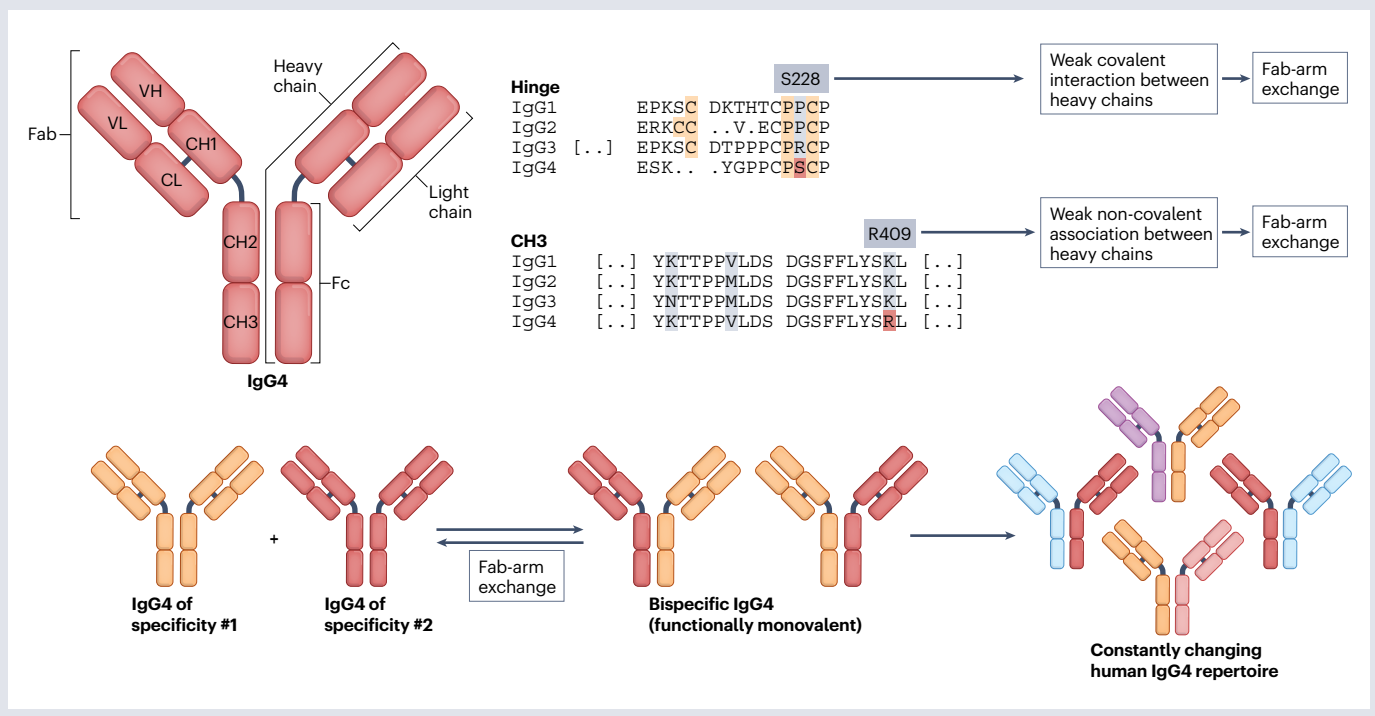
Fab-arm exchange

IgG antibodies are typically symmetrical molecules, with the exception of IgG4. IgG antibodies are covalent dimers of two half-molecules, each half consisting of a light chain and a heavy chain (see the figure). The antibodies produced by plasma cells are bivalent and monospecific (having two antigen-binding sites of the same specificity). However, human IgG4 is an unusually dynamic antibody, and these half-molecules can dissociate and recombine with other IgG4 half-molecules in a process termed Fab (fragment antigen binding)-arm exchange. The resulting IgG4 antibodies are asymmetrical, bispecific structures with two different antigen-binding sites. Owing to the heterogeneity of the IgG4 pool, up to 99% of circulating IgG4 is considered to be bispecific.

Two features of IgG4 enable Fab-arm exchange to occur: the serine at position 228 (S228) in the core hinge and the arginine at position 409 (R409) in the CH3 domain. The former results in unusually labile covalent interactions between the two heavy chains through disulfide bonds. The latter weakens the non-covalent association between the two heavy chains through their CH3 domains. Both features are required for Fab-arm exchange, as introducing only one of these residues in IgG1 does not result

in Fab-arm exchange *in vivo*. However, *in vitro*, where redox conditions can be varied more widely, a K409R mutant of IgG1 can be forced to participate in Fab-arm exchange in the presence of stronger reducing conditions than are typically required for IgG4 Fab-arm exchange. This forms the basis of the Duobody platform, which enables the production of bispecific therapeutic antibodies through *in vitro* Fab-arm exchange that are stable *in vivo*. For the other human IgG subclasses, particularly IgG3, the interactions between CH3 domains are also a lot weaker than for IgG1, but this is compensated by multiple disulfide bonds between heavy chains that are not present in IgG4.

A functional consequence of Fab-arm exchange is the effective monovalency of IgG4. The random nature of the exchange process results in the antigen specificity of one Fab arm recombining with any number of other, usually unrelated, antigen specificities in the other Fab arm. This eliminates the potential of the antibody to cross-link antigen, further minimizing the immune-activating potential of IgG4. Complement activation also seems to be further impaired by Fab-arm exchange³⁹. Fc, fragment crystallizable. Figure adapted from ref. 4, © Georg Thieme Verlag KG.



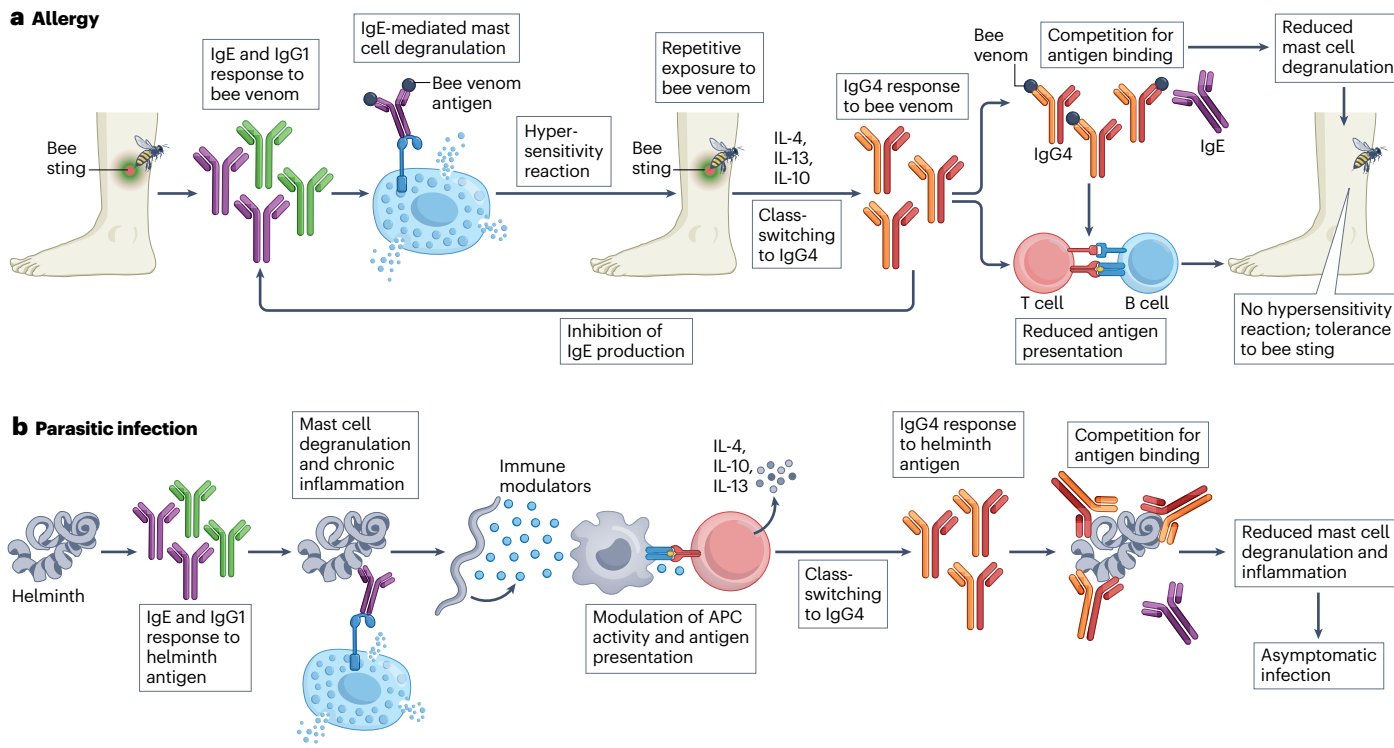


Fig. 3 | Physiological and potentially beneficial effects of IgG4. The effects of IgG4 can be beneficial to the host. **a**, In the case of allergy, repeated exposure to an allergen such as bee venom may at first elicit an antibody response dominated by IgE and IgG1, which stimulate inflammation and mast cell degranulation, causing a hypersensitivity response. Continued exposure to the same allergen may result in an increase in type 2 cytokines such as IL-4 and IL-13 and in regulatory cytokines such as IL-10, which stimulate class-switching to IgG4. The increased amounts of IgG4 compete with IgE for binding to allergen, thus preventing mast cell degranulation and inhibiting antigen presentation

to T cells by IgE on B cells, which blocks the inflammatory loop and results in tolerance. **b**, In parasitic infections, the IgE-mediated and IgG1-mediated inflammatory process can be detrimental to host tissue. Worms can secrete immune modulators that skew host cytokine production towards a type 2 profile (also including the regulatory cytokine IL-10), resulting in class-switching to IgG4 and, hence, obstruction of the inflammatory processes directed at the worm as well as reduced inflammatory damage to the host. Such events generally result in asymptomatic symbiosis. APC, antigen-presenting cell.

IgG4 levels⁷⁶. Interestingly, it has been suggested that a consequence of the IgG4-mediated attenuation of host immunity by the parasite may be protection of the host from autoimmune disease and allergies (see next section), although the role of parasitic infections in dampening allergy has not been unambiguously determined⁷⁷. Such a role would be consistent with the 'hygiene hypothesis', which suggests that lack of parasite exposure and the improved standards of hygiene in higher-income countries may cause increased prevalence of autoimmune and allergic diseases.

IgG4-dependent pathology

Specific deficiency of IgG4 can occur either as an isolated phenomenon (in ~30% of cases) or in combination with a deficiency of other antibody (sub)classes, such as IgG2, IgA or IgG1 (ref. 78). A selective lack of IgG4 or severely reduced levels of IgG4 are extremely rare. In some individuals (mostly children), IgG4 deficiency is associated with recurrent respiratory tract infections, allergies, candidiasis, chronic diarrhoea and chronic fungal infections^{78,79}. These observations suggest that IgG4 may have an unexplored physiological role in mucosal immunity. In line with this, a recent retrospective study observed IgG4 deficiency in ~20% of patients with inflammatory bowel disease, which was associated with

worse disease outcome⁸⁰. Future studies should elucidate whether there is a causal relationship between these observations.

IgG4 hypergammaglobulinaemia occurs in ~5% of the healthy population and seems without consequence. Several diseases are associated with pathogenic IgG4 responses (Fig. 4). Here, we discuss examples of pathology that are directly dependent on IgG4, including autoimmune diseases, antitumour responses and anti-biologic responses.

IgG4 autoimmune diseases

IgG4-AIDs were first defined as a separate subgroup of antibody-mediated autoimmune disorders in 2015 (ref. 10), and a first attempt at their classification based on the level of evidence for a pathogenic role of IgG4 was proposed soon thereafter^{11,81}. IgG4-AIDs are characterized by autoantibody responses predominantly of the IgG4 subclass against a known antigen. These disorders can affect many organ systems, depending on the major site of action of the targeted antigen, including the kidneys, central and peripheral nervous systems, haematopoietic system and skin. So far, a direct pathogenic role of IgG4 autoantibodies has been established for six IgG4-AIDs through passive transfer of IgG4 in experimental animals (Table 2), but this group is likely to expand

in the coming years with more evidence becoming available for 23 other candidate IgG4-AIDs. Diagnosis of an IgG4-AID is based on clinical symptoms and the detection of serum IgG4 autoantibodies to the disease-specific antigen. Antigen-specific IgG4 levels correlate closely with disease severity^{82,83}. As IgG4 is predominantly anti-inflammatory in nature and is not thought to induce pathology through Fc-dependent effector mechanisms, a key mode of action in all IgG4-AIDs is thought to be blocking essential protein–protein interactions of the target antigen¹⁰. For example, in muscle-specific kinase (MuSK) myasthenia gravis, which is a prototypical IgG4-AID, IgG4 autoantibodies to MuSK block its interaction with low-density lipoprotein receptor-related

protein 4 (LRP4), thereby obstructing a key trophic signalling cascade at the neuromuscular junction and resulting in fatigable skeletal muscle weakness⁸⁴. Much overlap between the IgG4-AIDs can be found in terms of their inflammatory status and treatment response. For example, B cell depletion therapy with rituximab often results in long-term remission in all of these diseases. These observations suggest that although IgG4-AIDs can present with various symptoms depending on the target antigen, they share an underlying immunophenotype.

Serum levels of IgG4 are, if at all, only slightly increased in patients with IgG4-AIDs (refs. 85–87) and the numbers of IgG4⁺ plasma cells and IgG4⁺ B cells are normal in circulation (M.G.H., unpublished

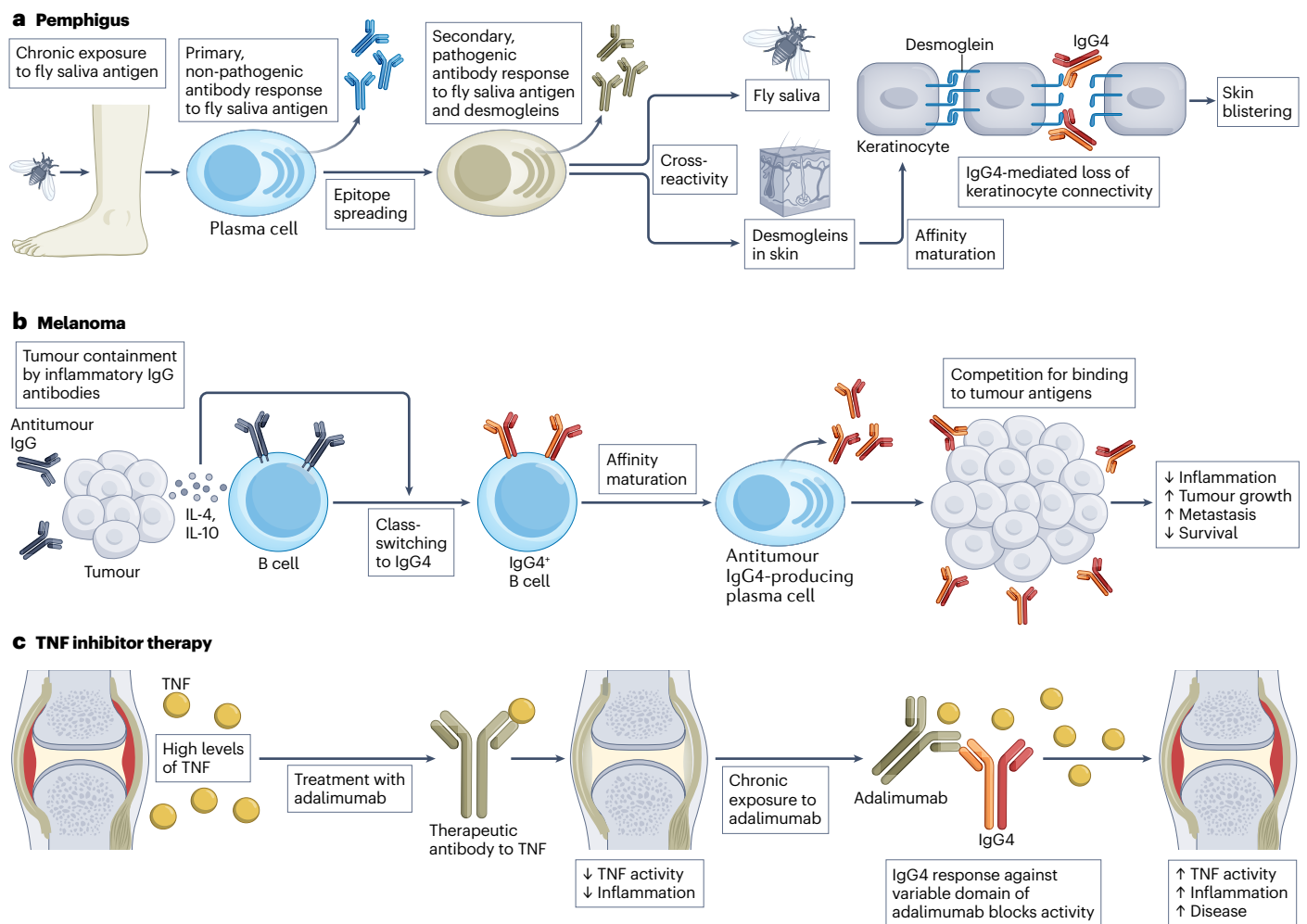


Fig. 4 | Pathological effects of IgG4 in three IgG4-associated disease settings. **a**, Pemphigus is one of the first-described IgG4 autoimmune diseases (IgG4-AIDs). There are indications that for some forms of pemphigus, exposure to a fly antigen causes an initial, non-pathogenic antibody response that is found also in asymptomatic individuals. In some individuals, epitope spreading occurs and a secondary, pathogenic antibody response develops in which the antibodies cross-react with desmogleins, proteins that are crucial for skin cell adhesion. Further affinity maturation of the autoimmune response results in high-affinity, predominantly IgG4 antibodies that physically obstruct the interaction of desmogleins with other cell adhesion molecules, leading to skin blistering. In some cases, the high-affinity IgG4 antibodies to desmogleins still cross-react with the fly antigen. **b**, In melanoma, tumour growth can be obstructed by IgG

antibodies that cause inflammation and destruction or growth inhibition of tumour cells. Some tumours, in turn, produce IL-4 and IL-10, which stimulate class-switching of local B cells and increased IgG4 production. High levels of high-affinity IgG4 compete with other antibody (sub)classes for binding to the tumour and prevent further inflammation and tumour destruction owing to their anti-inflammatory nature, leading to increased disease progression. **c**, In patients with chronic inflammatory diseases such as rheumatoid arthritis, the tumour necrosis factor (TNF) inhibitor adalimumab is often used to reduce inflammation. In some individuals, continuous exposure to adalimumab induces an IgG4 response against the variable domain of the therapeutic antibody, which thereby blocks the activity of the biologic and limits its therapeutic efficacy.

Table 2 | IgG4 autoimmune diseases with evidence by passive transfer of a pathogenic role for IgG4 autoantibodies

Disease (ORPHAcode)	Symptoms	Antigen (main epitope)	Target organ	HLA association	IgG4 abundance relative to total IgG	Response to anti-CD20 treatment	Pathological mechanism	Refs.
Chronic inflammatory demyelinating polyneuropathy (2932)	Proximal muscle weakness, distal sensory disturbances and areflexia; nephrotic syndrome in some patients	CNTN1 (N-terminal immunoglobulin-like 1 domain)	Axoglial junction (in complex with CASPR1 and NF155)	ND	>90%	Good	Blockade of NF155–CNTN1 interaction, resulting in loss of connectivity between axon and glial cells and impaired neuroconduction	140–143
Chronic inflammatory polyneuropathy (2932)	Proximal muscle weakness, distal sensory disturbances, and low-frequency and high-amplitude tremor with cerebellar features	NF155 (third fibronectin type III domain)	Axoglial junction (in complex with CASPR1 and CNTN1)	DRB1*15, DQB1*06	>90%	Good	In vivo, IgG4 depletes cell surface NF155; in vitro, IgG4 induces NF155 clustering but not internalization	139,140,142, 144–147
Myasthenia gravis (589)	Fluctuating fatigable skeletal muscle weakness	MuSK (N-terminal immunoglobulin-like 1 domain)	Neuromuscular junction (skeletal muscle)	DQB1*05, DRB1*14, DRB1*16	>90%	Good, often results in long-term remission	Obstruction of MuSK–LRP4 interaction, leading to AChR declustering, synaptic disintegration and neuromuscular transmission failure	84,148,149
Pemphigus foliaceus (79481) and pemphigus vulgaris (704)	Skin blistering	Pemphigus foliaceus: DSG1 (cadherin-like domain 1 and 2); pemphigus vulgaris: DSG3, sometimes together with DSG1 (cadherin-like domain 1 and 2)	Keratinocytes in the skin	DQB1*03, DQB1*05, DRB1*04, DRB1*14	Vast majority is IgG4 followed by IgG1, then IgG2 and IgG3	Initial response good	Blockade of desmoglein interactions and loss of cell–cell interactions resulting in skin blistering; autoantibody binding also affects cytoskeletal rearrangements and cell adhesion	82,88, 150–153
Thrombotic thrombocytopenic purpura (9358)	Microangiopathic haemolytic anaemia; risk of neurocognitive deficits and cardiovascular events	ADAMTS13 (cysteine-rich spacer domain 3)	Vasculature	DRB1*11, DRB1*12, DRB1*15	Initial response dominated by IgG1, chronic and relapse phases dominated by IgG4; level of inhibition of ADAMTS13 correlates with IgG4 titres	Good	Blockade of binding and cleavage of vWF, resulting in multimers of vWF that bind platelets and cause microthrombi	88,101, 154–156

Other diseases that are suspected to be IgG4 autoimmune diseases, but with lower levels of evidence, are described in ref. 81. AChR, acetylcholine receptor; ADAMTS13, a disintegrin and metalloproteinase with thrombospondin motifs 13; CASPR1, contactin-associated protein 1; CNTN1, contactin 1; DSG1, desmoglein 1; LRP4, low-density lipoprotein receptor-related protein 4; MuSK, muscle-specific kinase; ND, not determined; NF155, neurofascin 155; vWF, von Willebrand factor.

observations). These observations do not support a hypothesis that these patients have an overall tendency to develop dominant IgG4 responses but, rather, support an antigen-driven aetiology. A recent systematic review reports strong associations of IgG4-AIDs with HLA-DQB1*05 and HLA-DRB1*14, suggesting that these haplotypes predispose to the development of IgG4-AIDs (ref. 88). This may occur through directing B cell development and cytokine production, or by facilitating antigen presentation. In the case of three archetypal IgG4-AIDs – MuSK myasthenia gravis, pemphigus vulgaris and thrombotic

thrombocytopenic purpura – increased serum levels of the IgG4-promoting cytokine IL-10 have been reported^{89–92}. Although some autoimmune diseases mediated by IgG1, IgG2 or IgG3 are associated with tumour development, this has thus far not been reported for IgG4-AIDs, and the aetiology of IgG4-AIDs is expected to be different from that of such paraneoplastic syndromes.

Interestingly, evidence for molecular mimicry resulting in an IgG4-dominated response to desmogleins in the skin epidermis is found in patients with endemic pemphigus foliaceus from Brazilian and

Glossary

Antibody-dependent cell-mediated cytotoxicity

(ADCC). An immune response induced by antibodies binding through their Fc (fragment crystallizable) domains to pro-inflammatory, activating Fc receptors on immune cells, causing the immune cells to lyse the antibody-bound antigen or cell.

Antibody-dependent cellular phagocytosis

(ADCP). An immune response induced by antibodies binding through their Fc (fragment crystallizable) domains to pro-inflammatory, activating Fc receptors on immune cells, causing the immune cells to phagocytose the antibody-bound pathogen or cell.

Antibody-dependent complement deposition

An immune response induced by high levels of antigen-bound pro-inflammatory antibodies, causing precipitation of complement factor C1q and subsequent induction of the complement cascade, resulting in lysis of the antibody-bound pathogen or cell.

Class-switch recombination

Process by which B cells edit their immunoglobulin heavy-chain constant region DNA to produce antibodies of a different (sub)class. This results in antibodies that have the same specificity but different effector functions.

Complementarity-determining regions

Loops within the variable domains of an antibody (or B cell receptor) that are highly variable (between antibodies), unique and interact with the antigen.

Fab-arm exchange

A stochastic process in which IgG4 half-molecules recombine with other IgG4 half-molecules, forming an antibody that is bispecific and thus functionally monovalent.

Fc receptors

Immune receptors specific for antibody (sub)classes that can activate or inhibit immune cells upon binding to the Fc (fragment crystallizable) portion of antigen-bound antibodies.

Fragment antigen binding

(Fab). A highly variable antibody segment that confers its specificity for antigen binding.

Fragment crystallizable

(Fc). A constant domain of an antibody molecule that mediates the immunological effector functions of the antibody, including antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and antibody-dependent complement deposition. Antibody classes are grouped based on structural and functional (amino acid) determinants in this part of the antibody.

Molecular mimicry

Pathogens and endogenous proteins may have highly similar structures or molecular features. The molecular mimicry hypothesis suggests that (auto)immune responses may derive from an initial (antibody) response to a pathogen that cross-reacts with an endogenous protein having a highly similar structure.

Paraneoplastic syndromes

Autoimmune diseases that develop as a result of an antitumour antibody response. Tumour cells often overexpress proteins normally present elsewhere in the body. Antibody responses directed at such tumour proteins may not only contain the tumour but also cause disease at the natural site of activity of the protein.

Specific immunotherapy

A treatment regimen to induce tolerance, based on regular exposure to ultra-low doses of antigen that induce a class-switch to anti-inflammatory IgG4.

Tunisian populations (Fig. 4a). In these patients, antibodies develop against a salivary antigen from flies that are cross-reactive with desmogleins^{93,94}. Moreover, monoclonal antibodies derived from patients with pemphigus vulgaris were shown to be cross-reactive with walnut antigen⁹⁵. Both walnuts and flies carry allergens that are known to induce IgG4 responses. These observations suggest that exposure to certain IgG4-inducing antigens in combination with a permissive HLA haplotype and an IgG4-promoting immune environment (such as increased IL-10 levels) might have a role in the development of certain IgG4-AIDs. However, it is unclear which factors ultimately cause the IgG4 skewing of these responses.

Autoantibodies of other (sub)classes can also be found in patients with IgG4-AIDs, although usually of much lower titres. For several IgG4-AIDs, the pathogenicity of IgG1, IgG3 and IgM autoantibodies has also been confirmed⁹⁶⁻¹⁰¹. The mechanisms by which these autoantibodies induce pathology may differ between antibody (sub)classes and target antigens, and could include complement-mediated tissue damage and antigenic cross-linking and internalization causing surface depletion. The functional consequences of IgG4 autoantibodies may also differ between diseases²⁵. The relative contribution of IgG4 to pathology compared with the contribution of other antibody (sub)classes has not been carefully delineated, but the effects of different antibody (sub)classes may function in parallel to increase disease severity. Low titres of IgG1 autoantibodies are also found in non-symptomatic relatives

of patients with pemphigus vulgaris, which suggests that having (low levels of) such antibodies alone is not sufficient to precipitate disease symptoms¹⁰². It furthermore suggests that there is a subclinical stage in these autoimmune diseases that, upon early detection, could allow for IgG4-AID onset to be prevented. The role of autoantibodies of other (sub)classes in the pathophysiology of IgG4-AIDs requires further investigation.

Interestingly, in autoimmune diseases mediated by IgG1, IgG2 or IgG3 autoantibodies, a switch to an IgG4-dominant response may be therapeutic. Passive transfer of an IgG4 monoclonal antibody targeting acetylcholine receptor (AChR) inhibited subsequent complement-mediated damage and cytotoxicity induced by IgG1 binding to AChR, thus preventing the onset of AChR myasthenia gravis in rhesus macaques¹⁷.

Antitumour responses

Antitumour antibody responses can contain or even eliminate malignancies by binding to tumour cells and stimulating ADCC, antibody-dependent complement deposition and/or ADCP. However, in 1977 a prospective study in patients with melanoma identified high levels of IgG4 as having negative effects on survival¹⁰³. It has since become evident that some malignancies evade host immune defences by inducing class-switching of the antitumour antibody response to IgG4. IgG4 competes with other antibody (sub)classes for binding to tumour antigens

and owing to its anti-inflammatory properties blocks the induction of antitumour immune responses¹⁰⁴ (Fig. 4b). In the absence of an immune response, tumour cells have increased ability to proliferate and metastasize, resulting in disease progression and decreased survival. Immune evasion through class-switching to IgG4 has been observed in patients with melanoma, cholangiocarcinoma, colon cancer, pancreatic cancer and glioblastoma (reviewed in ref. 105).

Both the total IgG4 level and the number of IgG4⁺ B cells can be increased in the serum of patients with malignancies and are a negative prognostic indicator^{103,106,107}. These factors are also increased in the tumour microenvironment. The development of antitumour IgG4 responses results from IL-4 and IL-10 production by tumour cells, which directs a modified type 2 response that stimulates class-switching to IgG4 (ref. 104) (Fig. 4b). Furthermore, some tumour microenvironments contain tertiary lymphoid structures with functional germinal centres and T_{reg} cells¹⁰⁵. Crosstalk between these chronic inflammatory structures and the tumour may induce increased expression of IL-10 by T_{reg} cells¹⁰⁴. Importantly, it is not yet understood why certain tumours are capable of inducing IgG4 responses whereas others are not. In addition, although the reactivity of serum IgG4 antibodies from patients with cancer to tumour cells, for example to melanoma cells, has been confirmed, the precise antigen specificity of these antibodies has not yet been delineated¹⁰⁸. Broader study is needed to evaluate whether IgG4 has a pathogenic role in other cancer types. Interestingly, in addition to the proposed role of IgG4 in blocking inflammatory responses, a pro-angiogenic IgG4⁺ B cell subset (CD49b⁺CD73⁺IL-10⁻) was recently identified¹⁰⁹. These B cells were increased in the serum of patients with melanoma and might facilitate tumour angiogenesis.

Anti-biologic responses

IgG4-skewed responses can also occur as a result of chronic exposure to biological therapies. Such responses have been described for clotting factors FVIII and FIX used for the treatment of congenital haemophilia A or haemophilia B^{110–113}, for interferon- β used for the treatment of multiple sclerosis¹¹⁴ and for the tumour necrosis factor (TNF) inhibitors adalimumab and infliximab used for the treatment of inflammatory disorders such as rheumatoid arthritis and Crohn's disease^{115,116}. For each of these anti-biologic responses, the primary result is that the therapeutic effect of the biologic is impaired (Fig. 4c). Not all biologics trigger an IgG4-skewed anti-drug response. For example, interferon- β induces prominent IgG4 skewing only in some of the patients who develop an antibody response, which argues against chronic exposure to the biologic being the sole determinant of IgG4 skewing¹¹⁴. It is unclear why certain biologics cause these responses and others do not.

Haemophilia A and haemophilia B are severe clotting disorders caused by an inherited deficiency of FVIII or FIX, respectively. First-line treatment in these patients is chronic replacement of these clotting factors using either plasma-derived or recombinant proteins. Approximately 30% of patients treated with FVIII replacement therapy develop inhibitory antibodies predominantly of the IgG4 subclass¹¹⁷. Low-affinity IgG antibodies to FVIII of all subclasses can be found in both healthy individuals and patients with haemophilia, but high-affinity, high-titre IgG4 blocking antibodies to FVIII are unique to patients^{65,111}. Furthermore, high levels of anti-FVIII IgG4 correlate with decreased efficacy of FVIII replacement therapy¹¹⁸. Although these patients may also have antibodies to FVIII of other subclasses, IgG4 antibodies seem to be particularly detrimental. Interestingly, acquired autoimmune haemophilia A is also associated with a dominant IgG4

response to endogenous FVIII, which would thus classify this form of haemophilia as an IgG4-AID (ref. 119). For unknown reasons, FVIII – both endogenous and exogenous – has the propensity to induce an IgG4 response.

Many inflammatory disorders, including rheumatoid arthritis and Crohn's disease, can be successfully managed with TNF inhibitor therapy. Monoclonal antibodies are immunogenic to varying degrees, depending, amongst other factors, on their extent of humanization. However, even so-called fully human antibodies, of which the TNF inhibitor adalimumab is an early example, contain parts that are unique and foreign to recipients, namely the complementarity-determining regions responsible for target binding. B cell and T cell epitopes will be present in the biologic that can drive the development of high-affinity, class-switched (IgG4) antibodies¹²⁰. Similarly, although FVIII is a human protein, it will be seen by the immune system as a partially foreign protein in patients with congenital haemophilia owing to genetic defects in endogenous FVIII. This explains the potential for developing high-affinity, class-switched antibodies, although it is unclear why there is a tendency for these responses to favour class-switching to IgG4.

IgG4-related diseases

IgG4-RDs are a heterogeneous group of inflammatory disorders characterized by massive influx of IgG4⁺ B cells in affected organs and increased serum levels of IgG4 (refs. 9,121,122). Similar to IgG4-AIDs, a wide variety of organs can be affected in IgG4-RDs, including the thyroid, pituitary gland, pancreas, lungs, kidneys, gastrointestinal system and vasculature, with symptoms varying according to the organ affected. Diagnosis of an IgG4-RD is based on the histopathological finding of an IgG4⁺ B cell infiltrate, resulting in swelling of the organ, storiform fibrosis and obliterative phlebitis in a tissue biopsy. In addition, increased serum levels of IgG4 and of IgG4⁺ plasmablasts are often a good biomarker for both diagnosis and monitoring disease progression¹²³; 70–80% of patients have increased serum levels of IgG4. IgG4-AIDs and IgG4-RDs are currently considered to be separate disease entities as there is no evidence for large-scale influx of IgG4⁺ B cells in the affected organs in IgG4-AIDs or for significantly increased serum levels of IgG4 (refs. 85,87). The typical histology observed in IgG4-RDs involving fibrosis and tissue damage does not seem to have a major role in IgG4-AIDs, although biopsy data in the latter are limited. Furthermore, the pathogenic role of specific IgG4 and IgG4⁺ B cells in IgG4-RDs remains enigmatic. BCR repertoire sequencing confirmed the clonal expansion of IgG4⁺ B cells in patients with IgG4-RDs, suggesting that some clones may contribute specifically to disease onset and progression¹²⁴, although their antigen specificity was not determined. Further research is needed to determine the similarities and differences between IgG4-RDs and IgG4-AIDs in terms of their pathology, aetiology, histology and clinical features.

There are three hypotheses to explain the role of IgG4 in the pathophysiology of IgG4-RDs. First, IgG4-RDs, similar to IgG4-AIDs, are caused by IgG4 antibodies targeting autoantigens in the specific organ that is affected. Second, patients with IgG4-RDs have a type 2-skewed inflammatory environment, for as yet unknown reasons, which triggers pleiotropic IgG4 responses and impaired homing of IgG4⁺ B cells. Third, IgG4 is present in IgG4-RDs simply to dampen an ongoing immune response and, as such, does not contribute to the pathology.

Clinical clues to support an autoimmune hypothesis in these patients are the responsiveness to immunosuppressants, chronic disease course, presence of autoantibodies and HLA type II associations.

Passive transfer of IgG1 and IgG4 from patients with IgG4-RDs can induce similar pathology in experimental animals¹²⁵. Although IgG antibodies to autoantigens (for example, nuclear antigens, lactoferrin, carbonic anhydrases II and IV, pancreatic secretory inhibitor, trypsinogens and annexin A11) have been found, none of these consistently correlates with an IgG4-RD (refs. 126–128). Furthermore, these autoantibodies are mainly of the IgG1 subclass and target intracellular proteins that are unlikely to be the initial autoimmune trigger owing to lack of accessibility. By contrast, and in keeping with the second hypothesis, patients with IgG4-RDs generally have increased IgG4 reactivity against environmental antigens, suggesting that increased levels of IgG4 may be the result of a pleiotropic activation of IgG4⁺ B cells independent of their antigen specificity¹²⁹. Type 2 cytokines are increased in the serum of patients with IgG4-RDs, which could fit with both the first and second hypotheses¹³⁰.

Given the diverse nature of immune cells infiltrating affected organs in patients with IgG4-RDs, some argue that the IgG4 is induced in response to chronic immune stimulation and does not contribute to the pathology. Indeed, passive transfer of IgG1 purified from a patient with pancreatitis reproduced disease in mice, whereas symptoms were markedly reduced upon co-transfer of IgG1 and IgG4 isolated from the same patient¹²⁵. Furthermore, in addition to IgG4, levels of IgE are often increased in IgG4-RDs (refs. 131,132). Upon treatment with rituximab, both IgG4 and IgE levels have a tendency to decrease^{131,133}. Interestingly, this was also observed upon treatment with abatacept, which interferes with T cell activation, albeit in a limited number of patients¹³⁴. Furthermore, dupilumab, which blocks the receptors for IL-4 and IL-13, has recently been considered for the treatment of IgG4-RDs (ref. 134). These studies point to a role of T cells (possibly T_H2 cells) and IL-4 and/or IL-13 in the pathogenesis of IgG4-RDs, as well as indicating an apparent lack of persistence of IgG4 (and IgE) responses in this disease setting, which, at least for IgG4, seems to be a more general phenomenon⁷. Future research should focus on determining the sequence of events that lead to the production of IgG4 and confirming or acquitting a pathogenic role for IgG4 in IgG4-RDs.

Some reports suggest an increased incidence of malignancies in patients with IgG4-RDs, through the effects of IgG4 on suppressing antitumour immune responses, although this is still a matter of debate^{135,136}. Having a tumour may also predispose to developing an IgG4-RD through cytokines secreted by the tumour that induce class-switching to IgG4 (ref. 137). Whether having one type of IgG4-associated disease (IgG4-AID or IgG4-RD) can lead to a second type of IgG4-associated disease should be further investigated. Case reports of co-occurrence of IgG4-AIDs with IgG4-RDs do exist but are generally rare^{138,139}.

Future directions

The role of IgG4 antibody responses in physiological and pathological settings is gaining increasing attention. Although, historically, the anti-inflammatory nature of IgG4 was associated with dampening ongoing immune responses, it is increasingly recognized that these antibodies can also cause pathology. The first steps towards understanding the pathological mechanisms underlying these IgG4-associated diseases have been taken, but little is still known regarding what triggers and maintains these IgG4 responses. There is a clear need to better understand how IgG4 responses are regulated. This knowledge could then form the basis for novel therapeutic strategies targeting these responses. Specifically, the aim would be to stimulate beneficial IgG4 responses, for example in allergy, or to inhibit IgG4 responses in

autoimmune diseases and antitumour and anti-biologic responses. Supporting these (pre)clinical ambitions will require better models to study the development of ‘natural’ human IgG4 responses (Box 1).

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

M.G.H. is co-inventor on muscle-specific kinase (MuSK)-related patents. Leiden University Medical Center (LUMC) and M.G.H. receive royalties from these patents. LUMC receives royalties on a MuSK ELISA. T.R. declares no competing interests.

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